REMARKS

Claims 1-6, 10-13, 23, 28-30, 40-42, and 75-76 are pending in the instant application. Claims 1-5, 23, 28-30, 40-42, 75, and 76 are withdrawn without prejudice for being directed to a non-elected invention. Claims 6 and 10-13 are currently under examination. Claims 6 is amended herein. Support for the amendment can be found throughout the application, for example in the claims as filed, in Figures 8-10, and on pages 83-84 which discuss Figures 8-10 in detail. Claims 77-83 have been added. Claims 77-79 are supported, for example, on page 84, line 20, and page 85, lines 8-9. Claims 80 and 81 are supported, for example, on page 88, lines 14-20 and in Figure 14. Claim 82 is supported, for example, on page 10, lines 4-6. Claim 83 is supported, for example, on page 53, lines 20-23. Claims 7-9 were previously cancelled. After entry of the amendment, claims 6, 10-13, and 77-83 will be pending and under examination.

Withdrawn Claim Objections

Applicant thanks the Examiner for the withdrawal of the objection to claim 8.

Withdrawn Objection to the Specification-- Sequence Compliance

Applicant thanks the Examiner for the withdrawal of the objection to the specification for non-compliance with sequence listing requirements.

Withdrawn Rejection under 35 U.S.C. §112, Second Paragraph for Indefiniteness

Applicant thanks the Examiner for withdrawal of the rejection of claims 6 and 10-13 for being indefinite for failing to particularly point out and distinctly claim the subject matter.

Withdrawn Rejection under 35 U.S.C. §102 for Anticipation

Applicant thanks the Examiner for withdrawal of the rejection of claims 6 and 10-13 for being anticipated by the cited art.

Rejection of Claims under 35 U.S.C. §112, First Paragraph for containing New Matter

The Office Action has rejected claims 6 and 10-13 for allegedly containing new matter for reciting detection of an alteration in the KRAS2 gene wherein the alteration 'encodes a G35A amino acid substitution', as recited in independent claim 6. However, with regard to the `G35A', the specification as originally filed clearly indicates that this term denotes a G to A nucleotide substitution at position 35 in the cDNA (where the A of the ATG start codon is position 1). Applicant has amended claim 6 to recite that the alteration is in the nucleotide sequence, not the amino acid sequence. Withdrawal of the rejection is respectfully requested.

Rejection of Claims under 35 U.S.C. §112, First Paragraph for Lack of Enablement

The Office Action has rejected claims 6 and 10-13 under 35 U.S.C. §112, first paragraph for not being enabled for does not reasonably provide enablement for the breadth of the method as claimed which encompasses analysis of samples in any subject organism, and the detection of a nucleotide difference that encodes a G35A amino acid substitution, as recited in claim 6. Claim 6 has been amended to recite a G35A nucleotide substitution. Withdrawal of the rejection is respectfully requested.

Rejection of Claims under 35 U.S.C. §103(a) for Obviousness

The Office Action has rejected claims 7, 8, and 10-13 under 35 U.S.C. §103(a) for being unpatentable over Schouten et al (2002) in view of Maire et al. (2002) and Lecomte (2002). Applicant respectfully disagrees.

The Office Action asserts that with regard to the limitations of claim 6, Schouten et al. (summarized in Fig.2 on p.4, and Fig 8 on p.11) allegedly teaches the general method claimed. Schouten et al does not specify the analysis of a G35A KRAS mutation, nor that a G35A KRAS mutation is indicative of a phenotype.

However, the Office Action asserts that the analysis of a G35A mutation in KRAS2 and its association with pancreatic cancer was well known in the art at the time the invention was made.

Further, the Office Action asserts that Maire et al overcomes the deficiency of Schouten by teaching the analysis of mutations in differentiating between pancreatic cancer and chronic pancreatitis.

Relevant to claims 6, 10, and 13, Maire et al is alleged to teach the analysis of G12D mutations in codon 12 of the KRAS2 gene, which is the same G35A mutation of the instant specification. In relation to claims 10 and 13, Maire et al is alleged to teach determination and monitoring of mutation level.

Relevant to the limitations of claims 11 and 12, Maire et al is alleged to teach that the presence of the KRAS2 G35A mutation is indicative of pancreatic cancer as opposed to chronic pancreatitis. With regard to the limitations of claims 11 and 12, the teachings of Maire et al. allegedly indicate that a mutation level of 0.0% (e.g. undetected KRAS2 mutation) is indicative of chronic pancreatitis (claim 11), and a mutation level of 100% (e.g. KRAS2 mutation detected in all samples from a subject) is indicative of pancreatic cancer (claim 12).

Applicant disagrees with the Office Action in regard to the alleged teachings of Maire et al in relation to mutation levels. Maire et al. does not teach mutation levels. Maire et al. considers mutation level to be a binary question—is a mutation present or is a mutation not present? This is supported by the Examiner's statement that no mutation is indicative of chronic pancreatitis and a mutation is indicative of cancer. However, this is also incorrect. Only 44% of those with cancer were found by Maire et al. to have a KRAS mutation, **indicating that 56% with cancer were not found to have a KRAS mutation**. **Moreover, 13% of those with chronic pancreatitis and without cancer were found to have a KRAS mutation.** Therefore, the absence of a KRAS mutation is not indicative that a subject is cancer-free in 56% of subjects, and the presence of a KRAS mutation is not indicative that a subject has cancer in 13% of subjects, when the subjects are population selected from those having or suspected of having pancreatic cancer or chronic pancreatitis.

The instantly claimed invention is directed to **determining a mutation level**. The mutation level (or % mutant) of KRAS2 is equal to the mutant KRAS2/(mutant

KRAS2+wild-type KRAS2) (see legend of Figure 8). The importance of the mutation level is neither taught nor suggested by Maire et al. which considers mutation frequencies in populations, not mutation levels in individuals. None of the references cited in the Office Action teach or suggest that the mutation level for a single subject sample could be a results effective variable.

Maire et al also teaches the problem of relying on the presence of a KRAS mutation alone for the differentiation between cancer and pancreatitis. In Maire et al., "KRAS mutations were found in 22 patients (47%) with pancreatic cancer and **in four control patients with chronic pancreatitis (13%)**" (see abstract, emphasis added) The inability of Maire et al. to detect mutations in all subjects is a result of the methods used, but also the nature of pancreatic cancer. In the background section of Maire et al. cites a study that only 75-95% of pancreatic cancers have KRAS mutations at codon 12. Further, Maire et al. notes that prior studies looking at the presence of KRAS mutations in pancreatic cancer possibly had falsely high sensitivities and specificities as they used healthy controls rather than those with chronic pancreatitis.

The prevalence of KRAS mutations in those with chronic pancreatitis is a substantial confounding factor in any detection method which only considers the presence or absence of a mutation. Maire et al. report a sensitivity of only 47% and a negative predictive value of 52%. The use of a detection method other than that used by Maire et al. may increase or decrease the number of KRAS mutations detected and the sensitivity and specificity of the results obtained. **However, the sensitivity and negative predictive value are limited in an assay that only looks at the presence or absence of a KRAS mutation as long as true KRAS mutations are present in samples from subjects with chronic pancreatitis, and KRAS mutations are not always present in samples from subjects with pancreatic cancer**.

To improve the clinical value of the KRAS based diagnostic method of Maire et al., which has a high specificity and low sensitivity, the KRAS diagnostic method is combined with a serum carbohydrate antigen 19.9 test which has a relatively high sensitivity, specificity, positive predictive value, and negative predictive valued 91%, 87%, 91% and 87%, respectively (see Table 1). However, it was also noted that

cholestasis, type 2 diabetes, or negative Lewis antigen status could result in abnormal serum carbohydrate antigen 19.9 levels (see abstract and page 553, second column, first paragraph). **Therefore, as with the KRAS test, the serum carbohydrate antigen 19.9 test was known to have limitations due to the nature of patient samples, rather than any particular testing method**. The two tests combined had a much greater sensitivity, 98% and a negative predictive value of 96%.

Therefore, Maire et al provides two imperfect tests that, when combined, provide a reliable test. As the imperfections in the test are due to the nature of the samples, e.g., chronic pancreatitis sufferers having KRAS mutations and negative Lewis antigen subjects having no serum carbohydrate antigen 19.9, modifying the type of test used to detect the analyte at lower levels would not alter the nature of the samples. There can be no motivation to use a more sensitive assay to detect KRAS mutations, such as the assay provided by Schouten et al., as it would not change the nature of the samples.

Section 2144 sets forth potential rationales for combining references.

> >II. < THE EXPECTATION OF SOME ADVANTAGE IS THE STRONGEST RATIONALE FOR COMBINING REFERENCES
>
> The strongest rationale for combining references is a recognition, expressly or impliedly in the prior art or drawn from a convincing line of reasoning based on established scientific principles or legal precedent, **that some advantage or expected beneficial result would have been produced by their combination**. *In re Sernaker,* 702 F.2d 989, 994-95, 217 USPQ 1, 5-6 (Fed. Cir. 1983). >See also *Dystar Textilfarben GmbH & Co. Deutschland KG v. C.H. Patrick*, 464 F.3d 1356, 1368, 80 USPQ2d 1641, 1651 (Fed. Cir. 2006) (emphasis added).

As the samples themselves cause the false positives, no “advantage or expected beneficial result would have been produced by their combination” of Maire et al. with a reference that teaches a detection method in which lower levels of KRAS can be detected.

Maire et al. teach a combination of two imperfect tests to provide a more reliable test. Modification of Maire et al. to use only a single test, as in the instantly claimed

invention, cannot be obvious in view of the cited art. Section 2144.04(II) of the MPEP states:

> B. Omission of an Element with Retention of the Element's Function Is an Indicia of Unobviousness
>
> **Note that the omission of an element and retention of its function is an indicia of unobviousness**. *In re Edge*, 359 F.2d 896, 149 USPQ 556 (CCPA 1966) (Claims at issue were directed to a printed sheet having a thin layer of erasable metal bonded directly to the sheet wherein said thin layer obscured the original print until removal by erasure. The prior art disclosed a similar printed sheet which further comprised an intermediate transparent and erasure-proof protecting layer which prevented erasure of the printing when the top layer was erased. The claims were found unobvious over the prior art because the although the transparent layer of the prior art was eliminated, the function of the transparent layer was retained since appellant's metal layer could be erased without erasing the printed indicia.). (emphasis added)

Maire et al. rely on two essential elements, a KRAS mutation test and a serum carbohydrate antigen 19.9 test, to provide a clinically useful test to differentiate between pancreatic cancer and chronic pancreatitis. Applicant submits that alone a test having only 47% sensitivity and a 52% negative predictive value, as the KRAS mutation test, would not likely be considered to be clinically useful. Adding the serum carbohydrate antigen 19.9 test to the KRAS mutation test provides a test with good sensitivity, specificity, and positive and negative predictive value. The combination of the results from the two tests are needed to provide a reliable result. The methods of the instant claims, by determining not just the presence or absence of a KRAS mutation, but by further determining the mutation level of KRAS, allow for "the omission of an element", the serum carbohydrate antigen 19.9 test, "and retention of its function" providing a test with sufficient specificity and sensitivity to be clinically useful.

Further, even if Maire et al. suggested that detection of the mutation level of KRAS would be useful, which it does not, the quantification provided by Schouten et al. does not teach or suggest the sensitivity demonstrated in the instant application. The claimed cut-offs could not be considered to be taught or suggested by either of the references. For example, on page 8 of 13, first column, Schouten et al. teaches that

> The excellent reproducibility of relative signals obtained enabled the detection of a single extra copy of a probe target sequence per diploid genome.

That is, a 50% increase in copy number could (surprisingly) be detected.

The method of Schoten designed predominantly to detect large deletions (1N) or additions (3N) in genomic DNA, where the normal copy number is 2N. This is accomplished by ligating oligonucleotides to different regions of the genome, PCR amplifying them using tailed fluorescently labeled primers, and measuring the relative concentration of amplicons using capillary or gel electrophoresis. It can be used to detect 3 copies of chromosome 21 (Down's syndrome) or the X chromosome, whole exon deletions of BRCA2, exon losses of the mismatch repair gene hMLH1 and hMSH2, loss of the tumor suppressor gene p16/cdkn2b, extra copies (amplification) of erbb2, and germline mutation of the cystic fibrosis gene, cftr.

Detection in gains and losses in chromosomal regions refer to increases of 1.5-6.5 fold (page 9 of 13, second column-page 10 of 13, first column). A point mutation is detected in a heterozygote carrying a CFTR mutation that causes cystic fibrosis. The results from the assay are shown in Figure 8. The inequality in the size of the wild-type and F508 mutant peaks, when the sequences would be expected to be present in identical amounts, would suggest to one of skill in the art a limit on the accuracy of the determination of relative quantities of nucleic acid sequences.

In the Discussion section, possible applications are considered by Schouten et al. (see page 12 of 13, second column). All of the applications consider fold changes in the amount of a particular sequence present. Based on the teachings of Schouten et al., one could not expect the claimed cut-offs could be useful or even detected using the method provided therein.

In contrast, the method provided in the instant application is a conversion technology where a base substitution mutation is converted into a piece of DNA that is not part of the human genome (e.g. a part of the bacterial genome, LacZ). The converted DNA is then detected in realtime (Q-PCR) using a probe directed at LacZ.

Therefore, even if one were motivated to combine the methods of Maire et al. and Schouten et al., which one would not be, one would not arrive at the instantly claimed invention. A more sensitive method of detection of KRAS would not change the fact that cells containing mutant KRAS are present in samples from subjects with chronic pancreatitis.

Withdrawal of the rejection is respectfully requested.

Applicant has further added dependent claims 77-79 to the sensitivity of the instantly claimed diagnostic method. Although there is motivation to improve the sensitivity and specificity of nearly any clinical test, the mere suggestion that a test should be improved does not provide a reasonable expectation of success that one could improve a test based on references cited. Provided with the teachings of the cited references, one would not know how to achieve the level of sensitivity claimed.

Applicant has added claims 80-81 to further define the P1 and P2 oligonucleotides used in the methods claimed. Examples of sequences that can be used are provided, for example, on page 9, lines 14-30.

The instantly claimed invention is further distinguished from Schouten by the method used to detect amplification products. Schouten relies on the length of amplification products to determine the presence or absence of a specific sequence (see abstract). Schouten specifically teaches against the use of q PCR, particularly multiplex qPCR (see page 1 of 12, second column). Figures 2, 6, and 17 provide methods for multiplex qPCR. The instantly claimed method relies on the conversion of two oligonucleotide sequences based on annealing to a specific target sequence in a way to allow for ligation of the two oligonucleotides. This ligation results in a conversion of the oligonucleotides into a new target including foreign DNA sequences not present in the sample. The amplification is performed on the converted product to detect the presence or absence of the mutation rather than detecting the mutation directly. This allows for the quantitative detection of a point mutation on which the instant invention relies.

Applicant submits that the newly added claims are not obvious in view of the cited art.

CONCLUSIONS

In view of the above amendments and remarks, Applicants believe the pending application is in condition for immediate allowance. However, if the Examiner believes that there are any outstanding issues in the case that could be addressed by telephone conference, the Examiner is encouraged to contact the Agent for Applicant listed below to discuss the matter.

PETITION AND FEE AUTHORIZATION

It is believed that there is no fee due with this response. However, if a fee is due, the Commissioner is authorized to charge any fees associated with this submission, or any other submission by this Firm in relation to the instant application, to our Deposit Account, No. 04-1105, Reference 62310(71699). Any overpayment should be credited to said Deposit Account.

Dated: October 7, 2009

Respectfully submitted,

Electronic signature: /Colleen McKiernan/
Colleen McKiernan, Ph.D..
Registration No.: 48,570
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(617) 517-5555
Attorneys/Agents For Applicant